

3' to the carboxy terminus or 5' to the amino terminus of the first sequence, with the flanking sequence having an amino acid sequence substantially corresponding to that found on native HIV I gp41 adjacent said first sequence. In a preferred embodiment of the present invention, the monoclonal antibody ("5-21-3") is described, which is useful as a test reagent in diagnostic assays. In presently preferred forms, body fluid samples from patients are analyzed by immunoassay techniques such as radioimmunoassays, fluorescent immunoassays, or enzyme-linked immunosorbent assays in either direct or competitive formats.

Please replace the paragraph at page 23, lines 23 through 26, with the following replacement paragraph:

1) Synthesis of the peptide corresponding to gp41 amino acids 121-154 (SEQ ID NO:2).  
NH2-Asp-Arg-Glu-Ile-Asn-Asn-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-COOH

Please replace the paragraph at page 24, starting at line 28 and continuing through page 25, line 11 with the following replacement paragraph:

The polypeptide was purified by reversed-phase HPLC on C<sub>4</sub> columns, employing gradients of 0.1% TFA/water (A) and 100% acetonitrile (B) as the solvent systems at a flow rate of 1 ml/min for the analytical column (Vydac-214-TP54, Vydac Separation Group, Hesperia, California) or 3 ml/min for the semi-preparative one (Vydac-214-TP510).

The gradient used was:

28% B      1min      28% B      20min      47% B      1min      28% B

The polypeptide elution from the HPLC column was monitored at 225 nm and 280 nm. The composition of the polypeptide was confirmed by hydrolysis in 6 N hydrochloric acid (HCl)/0.3% phenol at 150°C for 2 hr in vacuo, and subsequently analyzed on a Beckman 6300 amino acid analyzer with a SICA 7000 A integration.

2) Synthesis of the peptide corresponding to gp41 amino acids 126-162 (SEQ ID NO:3).  
NH2-Tyr-Asn-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Try-Ala-Asn-Leu-Try-Asn-Trp-Leu-COOH

*A4*  
Please replace the paragraph at page 25, beginning at line 11 and continuing through page 26, line 3 with the following replacement paragraph:

This sequence of the peptide was assembled on the solid support by essentially the same procedure described above. The amino acid tryptophan was protected by the formyl (CHO) group. Double coupling protocols were used for amino acids underlined in the sequence shown above. The desired peptide was deprotected and cleaved off as described for the first peptide except that the peptide-resin was treated with a mixture of 8.5 ml HF, 1.0 ml of p-cresol and 0.5 ml ethanedithiol. The cleaved peptide was extracted using 15% and 40% aqueous acetic acid. The crude peptide so obtained was analyzed and purified on a C<sub>4</sub> reversed-phase column as described above except the gradient used was:

35% B 1 min 35% B 20 min 70% B 2 min 35% B  
linear linear

3) Synthesis of peptide corresponding to gp41 amino acids 131-154 (SEQ ID NO:1).

NH<sub>2</sub>-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-  
Glu-Leu-Asp-Lys-COOH

*A5*  
Please replace the paragraph at page 26, lines 4 through 15 with the following replacement paragraph:

This peptide was assembled, cleaved, and purified as described for the first peptide, except that the following gradient was used for analysis and purification:

15% B 1 min 15% B 20 min 40% B 2 min 40% B 2 min 15% B  
linear linear

4) Synthesis of peptide corresponding to gp41 amino acids 67-154 (SEQ ID NO:4).

NH<sub>2</sub>-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Ser-Pro-Trp-Asn-Ala-Ser-Trp-Ser-Asn-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Asn-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asn-Lys-COOH

*Please replace the paragraph at page 27, starting at line 19 and continuing through page 28, line 10 with the following replacement paragraph:*

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*Q*  
The stock solution of the peptide thus obtained was reduced with 50 mM DTT at 40°C for 90 minutes. The solution was brought to room temperature, and then dialyzed in a spectrapor membrane (cutoff 6500-8000) against a 0.1 M ammonium acetate buffer, pH 8.1, for 48 hr. The buffer was changed twice. After a total of 72 hr of dialysis, the peptide solution was diluted 3-fold with 0.1 M ammonium acetate buffer, pH 8.1, and allowed to stand in air for 48 hr. A UV spectrum of this peptide solution in water showed a maxima at 276 nm with a shoulder at 289 nm. The peptide was further purified on a reversed-phase C<sub>4</sub> column, and the analyzed as described above, using the following gradient:

30% B 1 min 30% B 20 min 65% B 1 min 30% B

5) Synthesis of peptide corresponding to gp41 amino acids 58-130 (SEQ ID NO:5).

NH<sub>2</sub>-Thr-Val-Trp-Gly-Ile-Lys-Glu-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-Thr-Thr-Ala-Val-Pro-Trp-Asn-Ala-Ser-Trp-Ser-Asn-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Asn-Tyr-Thr-Ser-Leu-COOH

6) Synthesis of peptide corresponding to gp41 amino acids 131-175 (SEQ ID NO:6).

NH<sub>2</sub>-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Asn-Leu-Trp-Asn-Trp-Leu-Asn-Ile-Thr-Asn-Trp-Leu-Trp-Tyr-Ile-Lys-Leu-Phe-Ile-COOH

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